USE OF TONICIFYING AGENTS TO ENHANCE RECOMBINANT ADENO-ASSOCIATED VIRUS YIELD

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Patent Application No. 62/408,420, filed Oct. 14, 2016, the entire contents of which are incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The invention relates generally to methods for enhancing recombinant adeno-associated virus vector (rAAV) yield, and, more particularly, the invention relates to the use of tonicifying agents to enhance rAAV yield.

BACKGROUND

[0003] Adeno-associated virus (AAV) is a non-pathogenic, replication-defective parvovirus. Recombinant AAV vectors (rAAV) have many unique features that make them attractive as vectors for gene therapy. In particular, rAAV vectors can deliver therapeutic genes to dividing and non-dividing cells, and these genes can persist for extended periods without integrating into the genome of the targeted cell. Given the widespread therapeutic applications of rAAV, there exists an ongoing need for improved methods of rAAV vector production including methods to achieve high-titer rAAV vector yields. Previous attempts to improve the production of a variety of viral vectors have included the use of cell culture additives such as metals, trace supplements, salts, and others (See, e.g., Williams, J. Gen. Virol., 9(3): 251-5 (1970), Weinbauer et al., Limnology and Oceanography, 54(3): 774-784 (2009), Yang et al., Hepatology, 48(5): 1396-403 (2008), and U.S. Publication No. 20150353899). rAAV are often produced by means of a helper virus, such as adenovirus (AV). However, any helper virus is a contaminant that must be removed before the rAAV can be used in therapeutic applications. There is therefore also a need in the art to produce rAAV with as little contaminating helper virus as possible.

SUMMARY OF THE INVENTION

[0004] The invention is based, in part, upon the discovery that a host cell used in the production of recombinant adeno-associated virus vectors (rAAV) will produce increased amounts of rAAV and decreased amounts of helper virus when the osmolality of the cell culture media is increased via the addition of an ionic tonicifying agent, such as NaCl. The invention is also based, in part, on the discovery that a host cell used in the production of rAAV will produce decreased amount of helper virus when the osmolality of the cell culture media is increased via the addition of a non-ionic tonicifying agent such as sucrose. In one aspect, the invention provides a method for producing a rAAV comprising incubating a host cell in a culture media with increase osmolality. In another aspect, the invention provides a method for decreasing the amount of helper virus produced by a rAAV-producing host cell, comprising incubating the rAAV-producing host cell in cell culture media with increased osmolality due to supplementation with a non-ionic tonicifying agent such as sucrose. In another aspect, the invention provides a method for increasing the production of rAAV and decreasing the production of helper virus produced by a host cell, comprising incubating the host cell in cell culture media with increased osmolality due to supplementation with an ionic tonicifying agent such as NaCl. In yet another aspect, the invention relates to cell culture systems comprising a host cell capable of producing both rAAV and helper virus, and a cell culture media with increased osmolality due to supplementation with a tonicifying agent.

[0005] In one aspect of the invention, the tonicifying agent is ionic. It is contemplated that the tonicifying agent may be any ionic tonicifying agent compatible with mammalian or insect cell culture media. Exemplary ionic tonicifying agents include NaCl, KCl, $NaNO_3$, $NaHCO_3$, Na_2SO_4, Na_2HPO_4, NaH_2PO_4, $NaNO_3$, KNO_3, K_2SO_4, K_2HPO_4, KH_2PO_4, or KNO_3. In one embodiment, the tonicifying agent is NaCl. It is contemplated that, where the tonicifying agent is NaCl, it may be present at a concentration of at least 4.5 g/L (77.0 mM). It is further contemplated that NaCl may be present at a concentration of at least 6.5 g/L (111.2 mM), or at least 7.5 g/L (128.3 mM). It will be understood that other ionic tonicifying agents can be substituted for NaCl by substituting an equal osmolar amount of the alternative salt.

[0006] In another aspect of the invention, the tonicifying agent is non-ionic. It is contemplated that the tonicifying agent may be any non-ionic tonicifying agent compatible with mammalian or insect cell culture media. For example, the non-ionic tonicifying agents may be a sugar, including disaccharides and monosaccharides, such as sucrose, fructose, glucose, galactose, mannose, maltose, and trehalose. In one embodiment, the tonicifying agent is sucrose. It is contemplated that, where the tonicifying agent is sucrose, it may be present at a concentration of at least 6.8 g/L (19.9 mM). It is further contemplated that sucrose may be present at a concentration of at least 13.7 g/L (40.0 mM), 29.4 g/L (85.9 mM), or 41.1 g/L (120.0 mM). It will be understood that other non-ionic tonicifying agents can be substituted for sucrose by substituting an equal molar amount of the alternative non-ionic tonicifying agent.

[0007] It is contemplated that the osmolality of the culture media, when measured at the start of incubation of the host cell, will be 360 mOsm/kg or higher, 375 mOsm/kg or higher, or 400 mOsm/kg or higher. In one embodiment, the osmolality of the culture medium is sufficient to produce at least a 20% reduction in total helper virus production, relative to that produced by a host cell in a cell culture medium at 266 mOsm/kg. It is further contemplated that the reduction in total helper virus production may be 30%, 40%, or 50%. In another embodiment, the concentration of ionic tonicifying agent is sufficient to produce at least a 50% increase in total rAAV production, relative to that produced by a host cell in a cell culture medium at 266 mOsm/kg. It is further contemplated that the increase in total rAAV production may be 100%, 150%, or 200%. In one embodiment, the period of time the host cell incubated in the cell culture medium with increased osmolality is for at least 2 days. It is further contemplated that the incubation may be at least 3 days or about 4 days.

[0008] It is contemplated that the host cell may be a mammalian cell, for example, a HeLa, HEK293, COS, A549, BHK, or Vero cell. It is also contemplated that the host cell may be an insect cell, for example, a Sf9, Sf-21, Tn-368, or BTI-Tn-5B1-4 (High-Five) cell. In one embodiment, the host cell is a HeLa cell. It is contemplated that the host cell may comprise a heterologous nucleotide sequence